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Research Article

The Antinociceptive Effects of Hydrazinocurcumin

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Background Analgesics in clinical used have many side effects and are not always effective. Hence need for safer and more effective agents. Hydrazinocurcumin is an azole derivative of the natural product curcumin. It is reported to have antiangiogenic, antiplasmodial and cytotoxic activities.

Objective: The antinociceptive activity of hydrazinocurcumin was evaluated.

Methodology: Hydrazinocurcumin was synthesized by reacting curcumin with hydrazine at room temperature and a yield of 81 % was obtained. It was investigated for in vivo antinociceptive activity using the acetic acid induced-writhing test while central antinociceptive activity was investigated using the hot plate method.

Results: Hydrazinocurcumin (22.4mg/kg) reduced acetic acid induced writhing by 42.7%. Its activity was comparable to that of sodium salicylate (50mg/kg). It did not increase reaction times of mice on the hot plate after 30 minutes of administration but increased the reaction time after 60 minutes.

Discussion: The findings suggest that hydrazinocurcmin has peripheral and delayed centrally mediated antinociceptive activity.

Conclusion: Hydrazinocurcumin may be a potential lead compound for agents with analgesic effects.

Keywords: hydrazinocurcumin, antinociceptive, hot plate method, acetic acid writhing test

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1. Introduction

The term "analgesics" refers to drugs that are used to abolish the pain sensation. The two major classes of analgesics in clinical use are narcotic or opioid analgesics and non steroidal anti-inflammatory drugs (NSAIDs). NSAIDs act by inhibiting the enzyme cycloxygenase and are further sub-classified on the basis of their selectivity for the target enzyme. Analgesics and anti-inflammatory agents in current use have many toxic effects that limit their therapeutic usefulness (Krenzischek et al, 2003). NSAIDs are notorious for causing gastric ulceration and renal failure (Rodrígiez et al., 2007). Cyclooxygenase-2 (COX-2) inhibitory agents have, unfortunately, been recently shown to have serious cardiovascular toxicity (Stacy et al, 2012). The acute adverse effects caused by opiod analgesics include constipation, drowsiness, confusion, nausea and itching. Tolerance and dependence occur after chronic use (Zollner and Stein, 2007). Forty percent of the patients suffering from chronic pain are dissatisfied with the drugs used to manage their pain (Eriksen et al, 2003). Consequently, there is a continuous search for safer agents that have a low addictive potential.

Curcumin (**1**, **Scheme 1**) is natural product isolated from *Curcuma longa/domestica* Linn. It has a wide range of biological activities which include antiiflammatory, cancer chemoprevention and antimicrobial (Sharma et

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al, 2005). Its clinical use is limited by its poor oral bioavailability and rapid metabolism (Ravindranath and Chandrasekhara, 1980; Ravindranath and Chandrasekhara, 1981). Hydrazinocurcumin (**Figure 1**) is an azole derivative of curcumin and it has been shown to possess *in vitro* antiplasmodial (Mishra et al, 2008), anti-inflammatory (Flynn et al, 1991), antiangiogenic (Shim et al, 2002) and cytotoxic (Ishida et al, 2002) activities. It inhibits 5-lipoxygenase (LOX) and cyclooxygenase (COX) enzymes (Flynn et al, 1991; Selvam et al, 2005). Its ability to inhibit these two enzymes may be attributed to its pyrazole ring. Pyrazole containing compounds have been reported to have potent analgesic and anti-inflammatory properties (Sauzem et al, 2008).

The in vivo antinociceptive properties of hydrazino-curcumin have not been previously investigated. In this study, the in vivo antinociceptive activity of hydrazinocurcumin was investigated.

2. Experimental

2.1 Materials and Instrumentation

Technical grade curcumin and deuterated solvents for NMR were purchased from Sigma-Aldrich Chemicals, St. Louis USA. Hydrazine hydrate from BDH Chemicals, Poole, England was kindly donated by the Chemistry Department, University of Nairobi. Glacial acetic acid was obtained from Manigate agencies, Nairobi, Kenya. Sodium salicylate was purchased from Hopkin and Williams, Essex, England. Morphine hydrochloride (10mg/ml) vials were obtained from Macfarlan Smith Ltd, Edinburgh, United Kingdom. Aluminium backed pre-coated Alugram® SIL G/UV_{254} aluminium backed plates for TLC were obtained from Macherey-Nagel, Germany. Melting point determination was done using a Büchi B450 Melting point apparatus. UV detection was done using a Min UVIS 131200 machine from Desaga Sarstedt-Gruppe. The IR spectrum was recorded on a Shimadzu FTIR Prestige® machine in the 4000 – 500 cm^{-1} range. The sample was dissolved in deuterodimethylsulfoxide and the proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Varian Unity Spectrometer at 400 MHz and 100 MHz respectively. All chemical shifts were recorded in ppm.

2.2 Animals

Male Swiss albino mice weighing 25 – 35g were obtained from the Animal house of the Department of Pharmacology and Pharmacognosy, University of Nairobi. For the period of the study, the mice were maintained in cages at a controlled temperature (23 ± 2ºC) with a 12 hour dark/light cycle. They were fed on standard mice pencils and water *ad libitium*. The institutional guidelines for the Care and Use of Laboratory Animals of the Department of Pharmacology and Pharmacognosy, University of Nairobi were followed.

2.3 Synthesis of Hydrazinocurcumin

Hydrazine hydrate and curcumin were added to a flask containing acetic acid (30 ml). The reaction mixture was stirred at room temperature for 90hrs (**Scheme 1**). The reaction was monitored by TLC on silica gel with dichloromethane: ethyl acetate (8:2) as the mobile phase. The reaction was stopped by addition of water. The precipitate formed was rinsed with water and dried at room temperature for 3 days.

2.4 Acetic Acid induced writhing test

The protocol described by Collier et al. (1968) was used. The mice were divided into three groups with six mice each. The groups were pre-treated with an intra peritoneal injection of hydrazinocurcumin (22.4mg/kg), the vehicle (0.2 ml of 0.7% v/v Tween 80, 3% v/v ethanol in water) or the sodium salicylate (22.4mg/kg) half an hour before an intra-peritoneal injection of 0.6% acetic acid (60mg/kg). The number of writhes occurring during the 30 minute period after injection was observed and recorded. For scoring purposes, a writhing movement was defined as stretching of the abdomen accompanied by extension of at least one of the hind limbs.

2.5 The Hot Plate Assay

The mice to be used were screened by placing them on a hot plate maintained at 55ºC. Only mice which did not react within 10 seconds were used. The selected mice were divided into three groups of six animals each. The first group was injected intraperitoneally with morphine (10mg/ml), the second with hydrazinocurcumin (22.4mg/kg), and the third with the vehicle (0.2 ml of 0.7% v/v Tween 80, 3% v/v ethanol in water). The assay was done by placing the mice on the hot plate and recording their reaction times. For the purpose of this study the reaction time was taken as the time before paw licking or jumping off the plate. Mice that did not react within 40 seconds were removed from the plate to avoid tissue damage. The latency time was recorded 30 and 60 minutes after administration of the test compound.

2.6 Data analysis

All data was analysed using Microsoft Excel 2003 and Stata version 9.0 software. The number of writhing movements per group and the reaction times in the hot plate assay was expressed as mean ± standard error of mean (S.E.M). Percentage inhibition of writhing was calculated by dividing the difference in number of writhes between a group and the control by the number of writhes recorded for the control group. Longitudinal analysis was performed using univariate ANOVA to determine whether there was a statistically significant difference in writhing episodes and reaction times with time. P values of less than 0.05 were considered significant.

3. Results

Characterization of hydrazinocurcumin

The reaction yield was 81%.

The NMR spectroscopic data were as previously described by Shim et al, 2002.

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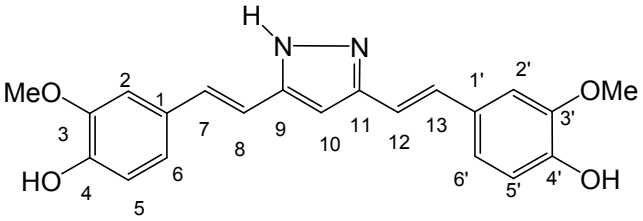
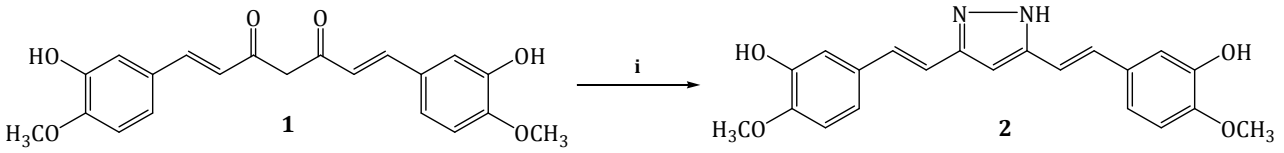


Figure 1: Structure of hydrazinocurcumin (**2**)

Pale yellow powder R_f = 0.33 (EtOAc/CH_2Cl_2 1:1) yield: 81 %, mp 217-218 ^oC (lit.[5] 211-212^oC); IR ν_{max} (KBr) /cm^{-1} 3685 (O-H), 3600 (N-H), 3063 (C=C-H), 2986 (C-H), 1757, 1713, 1606, 1447. ^1H NMR (400 MHz, DMSO-d_6): 3.81 (s, 6H, 2 X OCH_3), 6.60 (s, 1H, C_{10}-H), 6.76 (d, 2H, J = 8.1 Hz, C_5-H & $C_{5'}$-H / 7.35 (d, J = 8.5 Hz, C_5-H & $C_{5'}$-H) major/minor 4:1, 6.90 (d, 2H, J = 16.5 Hz, C_8-H, $C_{12'}$-H), 6.92 (dd, 2H, J = 8.1 Hz, J = 1.7Hz, C_6-H & $C_{6'}$-H), 7.03 (d, 2H, J = 16.5 Hz, C_7-H & $C_{13'}$-H), 7.12 (d, 2H, J = 1.7 Hz, C_2-H & $C_{2'}$-H), 9.12 (s, 2H, *Ar-OH*), 12.8 (br s, 1H, *N-H*). ^{13}C NMR (100 MHz, DMSO- d_6): 147.8 (C_4 & $C_{4'}$), 146.7 (C_3 & $C_{3'}$), 129.4 (C_7 & C_{13}), 127.6 (C_1 & $C_{1'}$), 119.9 (C_6 & $C_{6'}$), 115.6 (C_5 & $C_{5'}$), 109.6 (C_2 & $C_{2'}$), 99.2 (C_{10}), 55.6 (2 x OCH_3).



Scheme 1: Reagents and conditions. i) Hydrazine hydrate (1ml, 19.9mmol), curcumin (0.5g, 1.3573 mmol), glacial acetic acid (30 ml), room temperature, 90hrs.

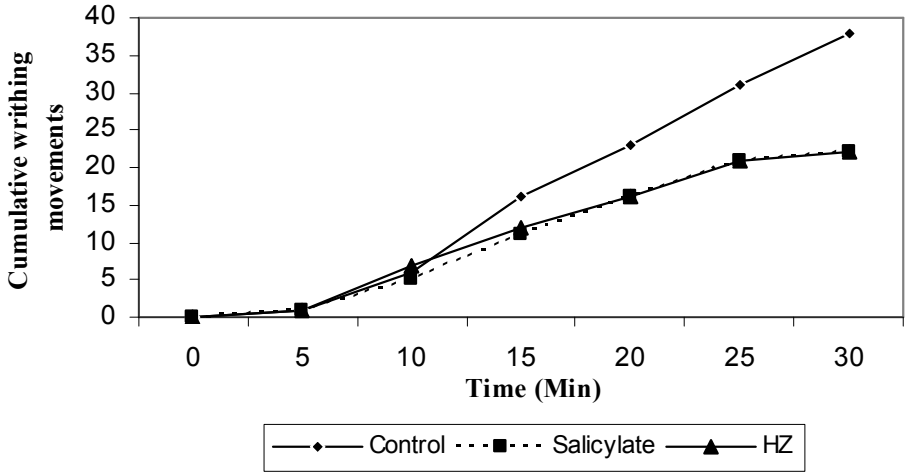


Figure 2: Effects of sodium salicylate and hydrazinocurcumin (HZ) on acetic acid-induced writhing in mice

Table 1: Hot plate assay - Effects of hydrazinocurcumin and morphine on mean reaction times of mice

Drug	**Mean reaction time (seconds)** (Mean ± SD)		
	Initial	**After 30 min**	**After 60 min**
Morphine	20.83 ± 0.83	36 ± 2.58	29.17 ± 3.75
Hydrazinocurcumin	21.58 ± 1.17	20.76 ± 1.28	28.68 ± 3.49
Vehicle (Control)	22.33 ± 2.22	16.67 ± 0.42	16.33 ± 0.56

Antinociceptive activity

Both hydrazinocurcumin (22.4mg/kg) and sodium salicylate (50mg/kg) inhibited acetic acid induced writhing by 42.7%. The cumulative number of writhing responses of mice treated with sodium salicylate and hydrazinocurcumin were very similar (**Figure 2**). In the hot plate assay, morphine substantially prolonged reaction times of the mice compared to hydrazinocurcumin (**Table 1**). It was noted that after

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30 minutes after drug administration the mean reaction time of mice treated with hydrazinocurcumin was on average 4 seconds longer than the response time of the control group but this was not statistically different from the response times of the control group (P=0.274). At one hour, the response time of hydrazinocurmin was comparable to that of morphine and this was statistically different from that of the control group (P<0.0001).

4. Discussion

The identity of hydrazinocurcumin was confirmed from its NMR spectrum and melting point which were similar to that reported in literature (Flynn et al, 1991; Shim et al, 2002). In the acetic acid-induced writhing assay, hydrazinocurcumin and sodium salicylate had comparable antinociceptive activities while in the hot plate assay, the activity of hydrazinocurcumin was no different from that of the control over the first 30 minutes. However it was noted that after 60 minutes of drug administration the reaction time of hydrazinocurcumin treated mice was longer than that of the control group. The acetic acid writhing test is an assay for agents with peripheral analgesic activity. The test is not specific for agents with peripheral analgesic activity and some agents devoid of analgesic activity such as clonidine and haloperidol show pronounced activity (Collier et al, 1968). The hot plate assay on the other hand is a fairly reliable assay for centrally acting analgesics. The findings seem to suggest that hydrazinocurcumin has peripheral antinociceptive activity that is comparable to that of salicylates. This activity may be attributed to its ability to inhibit COX and LOX enzymes (Flynn et al, 1991; Shim et al, 2002). It may have delayed centrally mediated antinociceptive activity because it delayed the reaction at 30 and 60 minutes after drug administration. This requires further investigation.

5. Conclusion

Hydrazinocurcumin (22.4mg/kg ip) has peripheral antinociceptive activity comparable to that of sodium salicylate (50 mg/kg ip). It may have delayed centrally mediated antinociceptive activity. These activities make it a potential lead compound for the development of newer analgesics agents.

Conflict of Interest declaration

The authors declare no conflict of interest

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